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ORAL DIURETIC ACTIVITY OF SRI LANKAN LOW GROWN ORANGE PEKOE GRADE BLACK TEA (*CAMELLIA SINENSIS* L.) IN RATS

WD Ratnasooriya*, TBS Muthunayake, EK Indeesha, CDT Ratnasooriya
Department of Zoology, University of Colombo, Colombo-03, Sri Lanka

***Corresponding Author:** Dr. Ratnasooriya WD, Professor, Department of Zoology, University of Colombo, Colombo-03, Sri Lanka.

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Abstract: In Sri Lankan ethnomedicine, black tea is claimed to be a diuretic. However, the grade of the tea is not specified. The aim of this study was to investigate the diuretic potential of Sri Lankan low grown orthodox whole leaf grade black tea (*Camellia sinensis* (L) O. Kuntz ; Family: Theaceae) using Orange Pekoe (O.P.) grade tea. Different doses of black tea brew (BTB) of O.P. grade tea [111 mg/kg (equivalent to 0.75 cups, N=7), 223 mg/kg (equivalent to 1.5 cups, N= 15), 446 mg/kg (equivalent to 3 cups, N=15) and 1339 mg/kg (equivalent to 9 cups, N=12)] or water (control, N=16) or Furosemide (reference drug, N=15) were orally administered to previously starved (18h) but subsequently hydrated (15 ml of isotonic saline) rats and their cumulative urine output was monitored at hourly intervals for 6h. The results showed moderate (compared to Furosemide), dose dependent diuretic activity. The onset of the diuretic activity was very rapid (within 1h) with an equally rapid peak dieresis (1-2h), but with a short duration of action (up to 2h). EC_{50} value for the diuretic action was 509.18 mg/kg. The diuretic action was accompanied with significant ($P<0.05$) increase in urinary Na^+ (Natiuresis), K^+ (Kaliuresis), pH, glomerular filtration rate (GFR) (in terms of creatinine clearance), aldosterone secretion index, thiazide diuretic index, intra and extra cellular pH regulatory index and urinary alkali index, whilst the carbonic anhydrase inhibition index was significantly reduced. In contrast, urinary Cl^-, and HCO_3^- levels, total dissolved solids, conductivity, density, and specific gravity remained unaltered. Overall, the results suggest that, Sri Lankan low grown O.P. grade black tea has moderate diuretic activity mediated via multiple mechanisms.

Key words: *Camellia sinensis*, Orange Pekoe Tea, Diuresis, Urine Output

INTRODUCTION

Most of the people enjoy tea on a daily basis. Besides water tea is the most consumed beverage of the world [(1)]. It is estimated that globally 3 - 5 billion cups, glasses or bowls of tea are consumed daily at present [(1)] with a per capita consumption of 2.52–3.16 kg/person/annum [(2)]. Sri Lankan daily consumption of tea is about 3.5 cups [(3)] accounting a per capita consumption of 1.38 kg/person/annum [(4)], which is 9-10% of world tea consumption [(5)]. Tea is manufactured from the two topmost leaves and buds of *Camellia sinensis* (L) O. Kuntz (Family: Theaceae) plant without any additives or preservatives in over 25 countries including Sri Lanka [(1)]. Based on the manufacturing technique there are three main types of tea: black (fully aerated or fermented), green (unaerated or unfermented) and oolong (semi aerated or semi fermented). Black tea accounts 78% of world's tea production and about 80% of global tea consumption [(1)]. Currently, Sri Lanka is the second main exporter and Sri Lankan black tea is drunk in more than 138 countries, accounting for 20% of global tea consumption [(6)]. Based on physical appearance of the manufactured black tea and its organoleptic properties (aroma, flavour, liquor, colour and taste) black tea is classified in to two main groups: whole leaf grade (twisted but not broken) and broken leaf grades.

In Sri Lankan traditional medicine tea is claimed to be a diuretic, [(7)] but the grade of tea is not specified. Recently, we experimentally showed that Sri Lankan orthodox high grown Dust grade No: 1 black tea [(8)] and Broken Orange Pekoe Fannings (B.O.P.F.) grade black tea belonging to all three major agroclimatic elevations [(9)] possess safe, mild to moderate oral diuretic activity supporting the ethnomedical claim. However, these two grades of black tea are broken leaf grades. On the other hand, diuretic potential of whole leaf grade black teas are not tested and investigation of this in rats using Sri Lankan low grown Orange Pekoe (O.P) grade black tea is the aim of the present study. The other aim is to assess the mode of diuretic action. Investigation of diuretic potential of Sri Lankan low grown O.P. grade black tea is worth examining since it is known that phytochemical composition and its bioactivities vary with several factors such as agroclimatic elevation, country of origin, tea brewing conditions, temperature of tea brew, processing method, age of leaf, harvesting season, particle size (grade of tea) of the manufactured tea [(10, 11, 12)].

MATERIALS AND METHODS

Experimental Animals:

Healthy adult Wistar rats of either sex (190 - 230 g) purchased from the Medical Research Institute, Colombo, Sri Lanka and bred in the animal house of the Department of Zoology, University of Colombo were used. They were kept under standardized animal house conditions (temperature: 28-31 °C, photoperiod: approximately 12 hours natural light per day, relative humidity: 50 - 55%) at the animal house of the Department of Zoology, University of Colombo. All the animals were acclimatized for 14 days before using for the experiment. All rats had free access to pelleted food (Master Feed Ltd., Colombo, Sri Lanka) and domestic tap water. All the experiments were conducted in accordance with the internationally accepted laboratory animal use and care guidelines [13]. Further, ethical clearance (Registration No: ERCIOB100/03/12) was obtained from the Ethical Review Committee of the Institute of Biology, Sri Lanka.

Source of Tea:

Topmost immature leaves and buds of *C. sinensis* plucked from the plantation of St. Jochims tea estate of the Tea Research Institute, Hedallana, Ratnapura, Sri Lanka (29 m above mean sea level: low grown) during November – December 2011 were used to process O.P. grade black tea by orthodox-rotovane technique at the estate factory. The composition of true to size particles defined for the O.P. grade black tea was determined using sieve shaker (Retsch AS 200, Retsch GmbH, Haan, Germany) with standard set of sieves (shaking time: 10 minutes and shaking speed: 50 vibrations/minute). Typical characters belonging to elevations were assessed organoleptically by professional tea tasters of the Tea testing unit, Sri Lanka Tea Board. Tea samples were packed in triple laminated aluminium foil bags (1 kg each) and stored at -20 °C until use.

Preparation of Black Tea Brew (BTB):

BTB was made according to the ISO standards; [14] adding 2g of O.P. grade black tea to 100 ml of boiling water and brewed for 5 min. This contained 36.1% (w/v) tea solids in water. Based on this data, 1339 mg/kg (equivalent to 9 cups, 1 cup = 150 ml) of BTB in 3 ml of water was prepared by adding 10 g of O.P. grade black tea to 30 ml boiling water and brewed for 5 min. Then 446 mg/kg (equivalent to 3 cups), 223 mg/kg (equivalent to 1.5 cups) and 111 mg/kg (equivalent to 0.75 cups) doses of BTB were prepared by diluting appropriately with boiling water.

Evaluation of Diuretic Activity:

Eighty rats were starved for 18 h and their urinary bladders were emptied by gentle compression of the pelvic area and pull of the tails. Each of the rats was then orally administered with 15 ml of isotonic saline (0.9% NaCl) to impose a uniform hydration status. One hour later, these rats were randomly divided into 6 groups and treated orally in the following manner; Group 1: 3 ml of water (N=16), Group 2: 111 mg/kg dose of BTB (N=7), Group 3: 223 mg/kg dose of BTB (N=15), Group 4: 446 mg/kg dose of BTB (N=15), Group 5: 1339 mg/kg dose of BTB (N=12) and Group 6: 13 mg/kg of Furosemide (N=15), the reference drug (State Pharmaceutical Corporation, Colombo, Sri Lanka). Each rat was then individually placed in a specially designed metabolic cage (Jayawardena Fabricators, Colombo, Sri Lanka) and their cumulative urine output was determined at hourly intervals for 6 h [8, 9]. The colour of the urine was also noted. During the experiment, rats had no access to food or water.

To investigate whether a tolerance developed to BTB induced diuresis with sub-chronic administration, 12 rats were randomly divided in to two equal groups (N=6/group). One group was orally administered with 3 ml of water daily (at 10.00 h) and the other group with 446 mg/kg dose of BTB of O.P. at the same time for 28 consecutive days. On 7, 14, 21 and 28 days of treatment, these rats were fasted and hydrated as described previously and they were individually placed in metabolic cages. Then their cumulative urine output was measured for 6 h.

Urine Analysis:

To investigate the mechanism of action of BTB of O.P. induced dieresis, 20 rats were randomly divided in to two equal groups (N=10/group) and fasted and hydrated as described previously. One group was orally administered with 3 ml of water and the other with 223 mg/kg dose of BTB of O.P. tea. These rats were then individually placed in a metabolic cages and their cumulative urine output was measured for 6h. The following urinary parameters were determined in collected urine samples: urinary Na^+ and K^+ levels (flame photometer, "Jencon PFP 7", Jencons Scientific Limited, Bedfordshire, UK), urinary H^+, Cl^- levels and pH (Ion Selective Meter, "Orion 720", Orion Research Inc., Boston, USA) and urinary HCO_3^- level (titrimetrically). Also, urine parameters such as glucose, specific gravity, protein, bilirubin, urobilinogen, ketone, nitrite, traces of blood and leucocytes were determined using 10M urine reagent strips (CYBOW, DFI Co., Ltd., Gimhae-City, Gyung-Nam, Korea).

Using urine output values obtained, the percentage of saline load excreted (volume of urine/volume of saline load X 100), percentage of urinary excretion (total urinary output/total liquid administered X 100), diuretic action (urinary output of treated group/urinary output of control group), and diuretic activity (diuretic action of treated test

extract/diuretic action of standard drug) were estimated [9].

Using the data obtained for electrolytes, the saliuretic indices for Na^+, K^+, and Cl^-; aldosterone secretion index (Na^+/K^+); thiazide diuretic index (Na^+/Cl^-); carbonic anhydrase inhibition index [$Cl^-/(Na^+ + K^+)$]; intra and extra cellular pH regulatory index (HCO_3^-/H^+), and urine alkali index (Na^+/H^+) were computed [9].

Estimation of Creatinine Clearance:

Ten rats were randomly divided into two equal groups (N=5/group), fasted and hydrated as described previously. One group was orally administered with 3 ml of water and the other with 223 mg/kg of BTB. These rats were then individually placed in metabolic cages and their cumulative urine output was measured after 2h and 24h. Blood was also collected from tail using aseptic precautions and serum was separated. Creatinine levels in the serum and urine were determined using Randox kits (Randox laboratories Ltd., Antrim, UK). Creatinine clearance was computed as per instructions given by the manufacturer using the above data. Creatinine clearance was taken as an estimation of the glomerular filtration rate [15].

Evaluation of overt signs of toxicity and behavioural abnormalities:

Twelve male rats were randomly divided into two equal groups (N = 6/group) and orally administered water or BTB of O.P. grade black tea daily (at 10. 00 h) for 28 consecutive days as follows; One group with 3 ml of water and the other with 446 mg/kg dose of BTB of O.P. grade black tea. During this period, each rat was observed for overt signs of toxicity (salivation, lachrymation, breathing distress, ptosis, stupor, squint, teeth exposure, rithing, convulsions, tremors, yellowing of fur, loss of fur), stress (erection of fur and exophthalmia), behavioural abnormalities (impairment of spontaneous movements, climbing, cleaning of face, ataxia, rolling and other postural changes), aversive behaviour (biting and scratching, licking of tail, paw and penis, intense grooming or vocalization) and diarrhoea [8].

Statistical Analysis:

Data are given as means ± standard error of the mean (SEM). Statistical comparisons were made as appropriate using one-way ANOVA followed by Tukeys family error post hoc test and Mann-Whitney U-test [16] using Minitab 14.0 statistical package. Significant level was set at P<0.05.

RESULTS

The sieve analysis of the sample showed that 83.5% of the tea particles were true size (1400 – 2000 µm) for O.P. grade black tea. Further, organoleptic profile of the tea tasters confirmed that the sample used be accepted as well made high quality low grown O.P. grade Sri Lankan black tea.

Evaluation of Diuretic Activity:

As shown in the Table.1 and Figure.1, 223 mg/kg dose of BTB (by 60%), 446 mg/kg dose of BTB (by 61%), 1339 mg/kg dose of BTB (by 38%) and Furosemide (by 138%) significantly (P<0.05) increased the 6h cumulative urine output. On the other hand, the lowest dose, 111 mg/kg although increased the urinary output by 32%, the effect was not significant (P> 0.05). The diuretic effect of BTB of O.P. grade tea was curvilinearly dose-dependent at each hour up to 6h [r^2 = 0.99 (at 1h), 0.91 (2h), 0.89 (3h), 0.77 (4h), 0.72 (5h) and 0.76 (6h); P< 0.05]. The EC_{50} value for the diuretic effect was 509. 18 mg/kg. Further, the 3 doses (1.5, 3 and 9 cups) which significantly (P<0.05) increased the cumulative urine output, also significantly (P<0.05) increased the % saline excreted and % urinary excretion. Interestingly, these 3 doses of BTB also exhibited a comparatively high diuretic action and diuretic activity (See Table.1). The onset of diuretic activity was very rapid (within 1h) and so was the time for peak diuresis (1 -2h) (See Figure.2). The duration of the diuretic action of BTB was short (up to 2h) (See Figure.2) whilst it was about 3h for Furosemide.

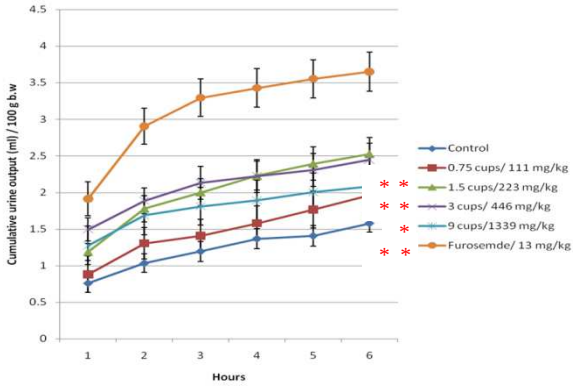

Figure.1: Cumulative hourly urine output (for 6 hours) of rats orally administered with 0.75 cups (111 mg/kg, N= 7), 1.5 cups (223 mg/kg, N= 15), 3 cups (446 mg/kg, N= 15) and 9 cups (1339 mg/kg, N= 12) with Sri Lankan low grown O.P. grade black tea (Mean ± SEM)

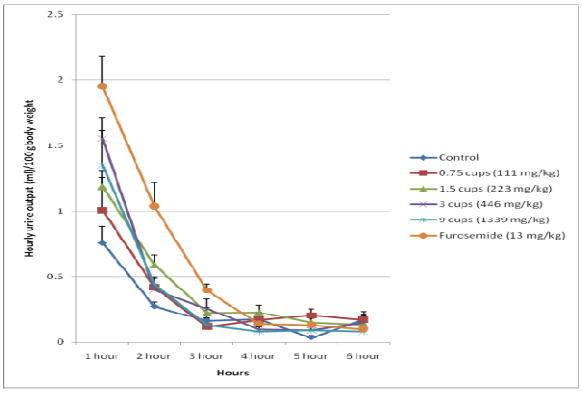

Figure.2: Hourly urine outputs (over 6 hours) of rats orally administered with 0.75 cups (111 mg/kg, N= 7), 1.5 cups (223 mg/kg, N= 15), 3 cups (446 mg/kg, N= 15) and 9 cups (1339 mg/kg, N= 12) with Sri Lankan low grown O.P. grade black tea (Mean ± SEM)

Table.1: Effect of oral administration of Sri Lankan low grown Orange Pekoe (O.P.) grade black tea on cumulative urine output of rats over 6 hours and on some selected diuretic indices

Treatment	Urine volume for 6h (ml/ 100g b.w.) (Mean ± SEM)	% Saline Excreted	% Urinary Excretion	Diuretic action	Diuretic activity	% increase of urine output at 6h
Control (n = 16)	1.578 ± 0.124	22.6 ± 1.66	18.83 ± 1.39			
0.75 cups (111 mg/kg) (n = 7)	2.092 ± 0.261	28.81 ± 3.02	24.01 ± 2.52	1.32	0.55	32.57
1.5 cups (223 mg/kg) (n= 15)	2.528 ± 0.219 **	34.57 ± 2.92 **	28.81 ± 2.43**	1.6	0.67	60.2
3 cups (446 mg/kg) (n= 15)	2.548 ± 0.213 **	35.06 ± 2.98 **	29.21 ± 2.48**	1.61	0.68	61.47
9 cups (1339mg/kg) (n=12)	2.184 ± 0.248 *	29.75 ± 3.05*	24.95 ± 2.54*	1.38	0.58	38.4
Furosemide (13 mg/kg) (n= 15)	3.760 ± 0.259 **	52.72 ± 4.14**	43.93 ± 3.45**	2.38	1	138.27

** - P < 0.01, * - P < 0.05 – compared to control (One-way ANOVA, followed by Tukey's family error post hoc test)
b.w. – body weight

With sub-chronic administration of 446 mg/kg dose of BTB, there was significant (P<0.05) enhancement of urine output (ml/ 100 g body weight) on day 7 (Control vs treatment: 1.66 ± 0.17 vs 2.64 ± 0.08), day 14 (Control vs treatment: 1.61 ± 0.12 vs 2.58 ± 0.11), day 21 (Control vs treatment: 1.58 ± 0.09 vs 2.72 ± 0.16) and day 28 (Control vs treatment: 1.56 ± 0.13 vs 2.55 ± 0.15). The cumulative urine output amongst these days was, however, not significant (P>0.05).

Urine Analysis:

The results are summarized in Table.2. As shown, 223 mg/kg dose of O.P. grade black tea significantly (P<0.05) increased the urinary Na^+ level (by 65%, marked natiuresis), urinary K^+ level (by 16%, moderate kaliuresis) and urinary pH (by 16%). In contrast, the remaining urinary parameters determined were either not significantly (P>0.05) altered [urinary Cl^- level (only by 9%), HCO_3^- level (only by 8%), total dissolved solids (only by 3%), conductivity (only by 6%), density (only by 0.4%), and specific gravity (only by 0.2%)] or not detected. Further, the colour of the urine of the treated rats appeared normal as that of control rats.

Of the urinary indices computed, (See Table.3) aldosterone secretion index (by 41%), thiazide diuretic index (by 51%), intra and extra cellular pH regulatory index (by 1411%) and urinary alkali index (by 2158%) were significantly (P<0.05) increased whilst the carbonic anhydrase inhibition index (by 26%) was significantly (P< 0.05) reduced.

The saliuretic index for Na^+, K^+, and Cl^- were respectively 1.65, 1.27 and 1.08. As shown, saliuretic index for Na^+ was markedly increased and K^+ was mildly increased. In contrast, saliuretic index for Cl^- remained almost unchanged.

Table.2: Effect of Orange Pekoe (O.P.) grade black tea on some urine parameters of rats (Mean ± SEM)

Parameter	Control	Treatment
Urobilinogen	0.1	0.1
Glucose	N	N
Bilirubin	N	N
Ketones	N	N
S.G	1.015 ± 0.001	1.017 ± 0.001
Blood	N	N
Protien	N	N
Nitrite	trace	trace
Leukocytes	N	N
Conductivity (mS/cm)	16.333 ± 0.544	17.371 ± 0.538
TDS (mg/l)	22646 ± 415	23252 ± 642
Na^+ (mg/l)	3612 ± 85.6	5956.6 ± 69.5 **
K^+(mg/l)	2097.5 ± 55.9	2440.3 ± 37.5 **
Cl^-(mg/l)	6.77 ± 0.22	7.37 ± 0.21
HCO_3^-(mg/l)	7178 ± 279	7724 ± 376
$H^+ \times 10^{-5}$(mg/l)	53.14 ± 4.76	6.75 ± 1.27 **
pH	6.29 ± 0.04	7.28 ± 0.11 **
Density (g/ml)	1.004 ± 0.005	1.000 ± 0.003

*- P < 0.05, ** - P < 0.01; compared to control (Mann-Whitney U-test)
N- Not detected

Table.3: Effect of Orange Pekoe (O.P). grade black tea on urinary indices of rats

Urinary index	Control (Mean ± SEM)	Treatment (Mean ± SEM)
Aldosterone secretion index (Na^+/K^+)	1.736 ± 0.069	2.447 ± 0.055 **
Thiazide diuretic index (Na^+/Cl^-)	538.2 ± 19.9	815.3 ± 30.6 **
Carbonic anhydrase inhibition index [$Cl^-/(Na^+ + K^+ (\times 10^{-3})$]	1.181 ± 0.034	0.874 ± 0.026 **
Intra and extra cellular pH regulatory index ($HCO_3^-/ H^+ (\times 10^5)$	149.7 ± 20	2262 ± 797**
Urine alkali index (Na^+ / H^+) ($\times 10^5$)	73.01 ± 6.61	1649 ± 548 **

*- P < 0.05, ** - P < 0.01; compared to control (Mann-Whitney U-test)

Evaluation of Creatinine Clearance:

The 223 mg/kg dose of BTB of O.P. grade black tea caused a marked (by 113%) and significant (P< 0.05) increase in glomerular filtration rate (GFR), as estimated by creatinine clearance at 2h post treatment (control vs treatment: 3.70 ± 0.85 vs 7.88 ± 1.27 ml/min).

Evaluation of overt signs of toxicity and behavioural abnormalities:

The 446 mg/kg dose of BTB of O.P grade black tea did not provoke any overt signs of toxicity, stress, behavioural abnormalities, aversive behaviours and diarrhoea.

DISCUSSION

This study examined the oral diuretic potential of BTB of *C. sinensis* made from Sri Lankan low grown O.P grade (a whole leaf grade of black tea) black tea in conscious rats using hydrated diuretic model. This is a widely used, reliable, sensitive validated and well recognized model for the assessment of diuretic potential of pharmacophores [17]. The tea sample used

was unblended, garden fresh and typical for the grade (in terms of sieve analysis and organoleptic properties). The results showed, for the first time, that Sri Lankan low grown orthodox O.P. grade black tea possess effective, acute, moderate (compared to furosemide and index of diuretic activity) and true (with marked natiuresis) diuretic activity (in terms of cumulative urine output, diuretic activity, diuretic action index, percentage saline excretion and percentage urinary excretion). Compared to Sri Lankan Dust grade No: 1[8] and B.O.P.F. grade [9] black tea which were previously shown to be diuretic, the order of diuretic property was O.P. > Dust No: 1 > B.O.P.F. This indicates that Sri Lankan whole leaf grade black tea has more diuretic action than broken grade black teas and the diuretic potential of black tea may depend on the size of the tea particles [9]. Further, it indicates it is more desirable for traditional practitioners to recommend O.P. grade tea in promoting diuresis. The diuretic activity of BTB of O.P. grade tea was dose-dependent suggesting that this effect is genuine, intrinsic, causal and have not been resulted from non-specific actions. Furthermore, with the sub-chronic administration of BTB of O.P. grade black tea, the therapeutic efficacy did not diminished indicating lack of development of tolerance.

On the other hand, with caffeine, the chief phytoconstituent of coffee, tolerance develops for its diuretic action [18]. Another feature of interest evident with the diuresis of BTB of O.P. was that it simultaneously increased the glomerular filtration rate (GFR) (in terms of creatinine clearance) and produced marked natiuresis (by 65%), moderate kaliuresis (by 16%) and extremely slight chlorouresis (by 9%). This indicates that BTB of O.P. acts both on glomerulus and on the uriniferous tubule in inducing diuresis. Such diuretic drugs are rare [19, 20, 21]. The diuretic action of BTB of O.P. had an extremely rapid onset (within 1h), equally quick peak diuresis time (1-2 h) but short duration of action (up to 2h). These observations suggest quick absorption of diuresis inducing phytoconstituents, possibly flavanols[1] including catechines[1], caffeine[1], thearubigins[1] and theaflavins[1] and that the diuresis is unlikely to be mediated via a secondary metabolite. The short duration of diuretic action suggest rapid metabolism and/or fast clearance of the active constituents. Such a diuretic profile is desirable in some forms of diuretic therapy. In complete contrast, in a preliminary study conducted in Sri Lanka by Gooneratna *et al.* [22] using a single dose of BTB (possibly made from blended black tea of Sri Lanka) has failed to show any diuretic activity (in terms of urinary volume, osmolality and urinary sodium, potassium levels). The lack of diuretic action in Gooneratna *et al* study may be due to usage of lower volume of weak aqueous infusion of tea or blended grades of black tea which could have very low potency. The results of this study together with our previous studies [8, 9] show marked differences in diuretic potency of Sri Lankan Orthodox black tea grades and interestingly, this depends on agroclimatic elevation and on the size of the tea particles [9].

The diuretic action of BTB of O.P. grade of tea was accompanied by marked natiuresis (in terms of urinary Na^+ level and sodium saliuretic index) and mild kaliuresis (as judged by urinary K^+ level and potassium saliuretic index) indicating that it exhibits true diuretic action and does not function as an aqueretic (agents that increase urinary output without promoting electrolyte loss) [23]. It is of interest to note that, several plants which are claimed to be diuretics in traditional medicine are indeed aqueretics [24, 25] and there are now synthetically available aqueretics such as tolvapton, lixivapton which are useful in certain clinical conditions [26].

Herbal extracts with high salt content is shown to produce diuresis[27]. But, such a non-specific mechanism is unlikely to be operative here as BTB of Sri Lankan tea has been shown to contain low salt content [28]. Some plant drugs stimulate the thirst center in the hypothalamus and thereby increases the fluid intake resulting in diuresis [27]. Such a mode of action is also unlikely in this study as the rats did not have access to water during the experimental period. BTB of O.P tea did not provoke an increase in urinary specific gravity, urinary density, urinary conductivity and total dissolved solids in the urine. The latter two parameters is an indirect measure of urine ionic content [29]. Collectively, these observations indicate that BTB induced diuresis cannot be attributed to an osmotic mechanism. Furthermore, therapeutically available osmotic diuretics are orally inactive and given intravenously [19, 21]. Interestingly, BTB of Dust grade No: 1 [8] and B.O.P.F. grade [9] tea were also not mediating their diuretic action via an osmotic mechanism. It is well known that ADH plays a pivotal role in regulation of water balance in the body [19, 20]. An impairment of ADH release from the posterior pituitary and/or an inhibition of its water re-absorption ability at distal convoluted tubule and/or collecting duct may induce diuresis in this study. Alternatively, BTB of O.P. tea could have an ADH antagonistic activity such as with new class of FDA approved drugs such as tolvoptan or lixivapton [26] But, such modes of actions are unlikely as there was no change in the urinary specific gravity, urinary density, urinary conductivity and total dissolved solids in urine. Further, inhibition of ADH causes polyuria with low osmolality [30] and currently available ADH antagonists require intravenous administration [26]. Some botanicals are claimed to induce diuresis, at least partly, by increasing peristalsis of ureters [17], number of functional glomeruli in kidneys [17] or by down regulation of mRNA of aquaporin – R, and vasopressin V_2 receptor as claimed for *Polyporus umbellantus* plant

[31]. However, at present, we do not have evidence in favour or against such potential mechanisms with BTB of O.P. but worth examining.

BTB of O.P. grade tea triggered a marked increase in glomerular filtration rate (in terms of creatinine clearance) which can obviously contribute to its diuretic action. An increase in renal blood flow and cardiac output due to caffiene [18] in BTB could be attributed for this mechanism of action.

Urine of BTB of O.P. grade treated rats was markedly hypernateremic (in terms of urinary Na^+ level and sodium saliuretic index), mildly hyperkalemic (in terms of urinary K^+ level and potassium saliuretic index) and was not hyperchloremic (in terms of urinary Cl^- level and chloride saliuretic index). Further, it elevated (by 51%) the thiazide diuretic index (Na^+ / Cl^-) significantly with a slight but significant alkalization of urine (which is not usually seen). Collectively, these observations suggest a thiazide like mode of diuretic action for BTB of O.P. tea. Thiazide type of diuretics increase thiazide diuretic (secretary) index and simultaneously increase urinay Na^+ and K^+ levels by inhibiting the Na^+ / Cl^- symporter co-transporter in the distal convoluted tubule of nephron [19, 33] by competing for Cl- binding sites and increasing excretion of Na^+ by inhibiting Na^+ reabsorption [19]. BTB of Dust grade No: 1 tea [8] and B.O.P.F. grade tea [9] also exhibited this mode of diuretic action.

In this study, BTB of O.P. tea also simultaneously provoked a huge and a significant impairment in urinary Cl^-/ $Na^+ + K^+$ ratio (an index if increased carbonic anhydrase inhibitory activity) and increase in the urinary HCO_3^-/H^+ ratio (cellular pH regulatory index). Collectively, these observations indicate an inhibition of carbonic anhydrase enzyme activity in the distal convoluted tubule [32] in inducing diuresis. This mode of diuretic action has been previously shown for BTB of B.O.P.F. grade tea [9].

BTB of O.P. grade tea significantly increased the urinary aldosterone index (urinary Na^+/K^+ ratio) and caused an alkalization of urine (in terms of urinary pH and alkali index). Further, the onset of diuresis was extremely rapid. However, the increase in urinary K^+ level was slight (16%) compared to urinary Na^+ level (65%). Collectively, this may suggest a weak potassium sparing diuretic mechanism which is of clinical relevance. Such a mode of diuretic action is not reported with BTB of Dust grade No: 1 [8] or B.O.P.F grade [9] or to any other grade of black tea.

On the other hand, in addition to what has been described previously, the diuresis provoked by BTB of O.P. was extremely rapid (1-2 h), moderately strong (in terms of cumulative urine output and diuretic action, diuretic action index, % saline excreted and % urinary excretion) with a marked increase in urinary Na^+ level (by 65%) and mild increase in urinary K^+ level (by 16%) and exhibited a diuretic profile (Figure:2) almost similar to furosemide, a loop diuretic which acts by inhibiting the $Na^+/K^+/Cl^-$ co-transporter in the thick region of the ascending limb of loop of Henle [19]. These observations suggest that BTB of O.P. may have a low ceiling loop diuretic type of mode of action [19, 21]. However, loop diuretics usually increase urinary Cl^- level but, in this study, there was no chlorouresis. Such a mode of loop diuretic action has been reported with *Ruta graveolens* leaves [15] and even furosemide has been shown not to always increase urinary Na^+ level in excerting its high ceiling loop diuretic action [33]. Loop mode of diuretic action is not reported with BTB of Dust grade No: 1 [8] tea or B.O.P.F. grade tea [9] or any other kind of black tea.

Interestingly, no overt signs of toxicity, behavioral abnormalities, stress and aversive behaviour were evident with sub chronic administration of BTB of O.P. grade black tea.

Diuretics are generally used in the treatment of hypertention, congestive heart failure, ascites, pulmonary oedema, nephritic syndrome, renal failure and pregnancy toxcemia [34], cirrhosis of the liver, oedema in pregnancy, and also to lower intraocular and cerebrospinal fluid pressure [36]. However, most have side effects such as causing fatigue, weakness, or impotence [34], causing electrolyte imbalance, development of new-onset diabetes and activation of the rennin-angiotensin-neuroendochrine systems [35]. Lack of these distressing side effects and potent diuretic activity (mediated by multiple mechanisms) in BTB of Sri Lankan orthodox black tea[(Dust No: 1) [8], B.O.P.F (9) and O.P (present study)] suggests that these could be used as an adjuant together with clinically used diuretics both in allopatric and traditional systems of medicine. Furthermore, this study and our previous studies [8, 9] scientifically support the claim in Sri Lankan traditional medicine that Black tea is diuretic.

CONCLUSION

This study shows for the first time that Sri Lankan orthodox low grown O.P. grade black tea of whole leaf grade type possesses moderate diuretic activity which is mediated by multiple mechanisms which is uncommon in the field of diuretics.

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